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Original Article

AKT2 GENE POLYMORPHISMS IN INDIAN WOMEN WITH POLYCYSTIC OVARIAN DISEASE (PCOD)

AAJI PASHA SHEK, V DEEPA SWITHA, CHAITANYA SRAVANTHI CHILLARA & SUJATHA MADIREDDI[#]

Institute of Genetics & Hospital for Genetic Diseases, Osmania University, Ameerpet Road, Begumpet, Hyderabad, Telangana, India

ABSTRACT

Polycystic Ovarian Disease (PCOD) has been reported to be the most prevalent endocrine condition among women in their reproductive age. About 70% of women with Polycystic Ovarian Disease (PCOS) have been reported to have insulin resistance. The AKT2 gene has been reported to be highly expressed in cells involved in the insulin, apoptotic as well as mitogenic signaling pathways. The aim of the research work was to determine the genetic interaction of the four AKT2 gene SNPs (rs2304188, rs11671439, rs3730051 and rs8100018), along with hormonal and clinical characteristics in PCOD patients in comparison to healthy individuals in the Indian female population. The study reports that rs11671439 CT and rs2304188 CT genotypes are significantly associated with the increased risk for the disease by 2.41 (95% CI=1.50-3.87) fold and 2.32 (95% CI=1.49-3.60) fold respectively. The rs11671439 T, rs8100018 G, and rs2304188 T minor alleles are significantly associated with the increased risk for the disease by 1.78 (95% CI=1.20-2.62) fold, 1.66 fold (95% CI=1.11-2.47) and 1.90 fold (95% CI=1.34-2.71) respectively. Genotype combination analysis revealed that the presence of multiple polymorphisms in the AKT2 genes might increase the risk for PCOD further in comparison to the presence of individual polymorphism.

KEYWORDS: PCOD, AKT2 & Infertility

Conflict of Interest: None

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INTRODUCTION

Polycystic Ovarian Disease (PCOD) has been reported to be the widely prevalent endocrine disorder among women in their reproductive age, reporting 6%-10% rate of prevalence (Shenoy & Brundha, 2016). The incidence rate for PCOD has been found to be varying from 5% to 10% and has been reported among women during their reproductive age across all ethnicities and cultures. An incidence of 9.13% for PCOD has been reported in a prospective study of Indian adolescents (Shenoy & Brundha, 2016). Among the predominant features observed among PCOD patients are anovulatory or irregular menstrual cycles having symptoms of seborrhoea, hyperandrogenism such as acne, alopecia, hirsutism, as well as polycystic ovaries. Studies have reported association of insulin-resistance (IR), obesity, as well as the possibility of developing Type 2 diabetes mellitus (T2DM) among females with PCOD (Shenoy & Brundha, 2016). Although many women perceive the disease as cosmetic due to the presence of hirsutism and acne, but there may be gynaecological problems causing irregular menstrual cycles and reduced fertility. The most important aspect of PCOD is the proper diagnosis of the disease and management of the patient. For the successful outcome of the treatment regime; changes in lifestyle and long term

medications are extremely essential. Investigations used for the diagnosis and treatment of PCOD include Laparoscopy, ultrasound scan, hysteroscopy and hormonal investigations among which ultrasounds are commonly used. PCOD treatment is mostly focused on the reported abnormalities as well as treatment for infertility among overweight women which includes food control, exercise, weight loss, and skincare (Chandrasekhar & Brundha, 2016).

About 70% of patients with Polycystic Ovarian Disease (PCOD) have been reported to have insulin resistance. The exact molecular function behind this molecular mechanism remains unclear. However, studies have reported the defects in the post-insulin receptor domain as a vital contributor to the onset of hyperandrogonemia as well as insulin resistance (Carmina & Lobo, 2004). There are two different pathways involved in the regulation of insulin signaling which includes phosphatidylinositol 3-kinase (PI3K) activation pathway as well as mitogen-dependent protein kinase activation pathway (Goodarzi, Jones, Chen, & Azziz, 2008). Various studies have reported the role of PI3K activation in the downstream molecules among patients with PCOS where molecular defects were identified in adipose and fibroblasts tissue among PCOS patients were found to be associated with deregulation in the post–insulin receptor signaling pathway (Diamanti-Kandarakis & Papavassiliou, 2006). The role of AKT (AKT serine/threonine kinase) gene in the activation of 3-phosphoinositide dependent kinase (PIDK) pathways has been well studied. The genes coding for the three AKT variants (AKT1, AKT2 and AKT3) are found to be highly conserved among which the gene for AKT2 are conserved in the 19q13.2 locus consisting of 22 exons. The AKT2 gene has been reported to be highly expressed in cells involved in the insulin, apoptotic as well as mitogenic signaling pathways (Azziz et al., 2009; Vanhaesebroeck & Alessi, 2000). Previous study has reported that the lack of Akt2 isoform which is necessary for insulin signaling leads to an increased level of testosterone as well as increased risk for ovarian cysts, which is also found among insulin-resistant PCOS patients (Restuccia, Hynx, & Hemmings, 2012). Studies have also reported that upregulations of PI3K-Akt, as well as ERK signalling, can cause molecular dysregulations which may lead to changes in hormonal and metabolic activity, which may lead to PCOS-like characteristics in animal models (Ryu, Kim, Kim, & Ku, 2019).

Based on previous reports and studies, the aim of the research work was designed to determine the genetic interaction of the four AKT2 gene SNPs (Single Nucleotide Polymorphisms), along with hormonal and clinical characteristics in PCOD patients in comparison to healthy individuals in the Indian population.

METHODS

Subjects

The study included 200 Indian patients with PCOD aged 18-35 along with age-and BMI-matched 200 Indian healthy disease free individuals as controls. The Body Mass Index (BMI) was estimated from the weight and height of the individuals. The PCOD patients included in the research work were included from the Institute of Genetics & Hospital for Genetic Diseases, Osmania University. The research work has been approved for research by the Institutional Ethical Committee for Biomedical Research. The ethical criteria for the study were followed as per the guidelines issued by the Institutional ethics committee. Patients who were found to be in their premenopausal stage, devoid of pregnancy symptoms as well as patients not undergoing any sort of hormonal therapy for minimum 3 months before the study were included in the study. The females of the control group consisted of healthy women who do not have any PCOD family history or irregular menstrual cycles. It was also ensured that the individuals of the control group did not undergo any hormonal therapy. All the information regarding the menstrual cycle, reproductive and gynecological history, use of medications, history of PCOD and any other associated disease were collected in the form of a questionnaire. Study subjects with diabetes were

excluded from the study. Signed informed consent for participation in the research work from all the study subjects was obtained.

Serum Measurements

Whole blood was collected in EDTA vials on the second and third day of the menstrual cycle from the female study subjects followed by centrifugation at 3500 rpm for 10 min. The obtained serum was stored at -80°C till further process. The level of LH, FSH, and TSH hormones were measured using automated immunochemical analyzer.

Isolation of Genomic DNA

Blood (200 µL) from the study subjects were collected in EDTA tubes which were used for isolation of DNA using the QIAamp DNA Blood Mini kit (Qiagen, Germany). The samples were collected. The quality and purity of the DNA were determined by fluorescence readings using Quant-iTdsDNA-BR kit in the Qubit 1.0 fluorimeter. The genomic DNA extracted was refrigerated at -20°C for future use.

Amplification using PCR

PCR was performed for the amplification of rs2304188, rs11671439, rs3730051 and rs8100018 *AKT2* genes using LightCycler 1.5 (Roche Applied Science, Switzerland). The reaction mixture for the PCR amplification had a 10 µL reaction volume which included ~30 ng genomic DNA, 2 pmol/µL each of forward primer and reverse primer (Supplementary Table 1), 1 U of Taq polymerase and 3 mM concentration of MgCl2. Nuclease free H_2O was used to adjust the volume to 10 µL.

Genotyping

Four *AKT2* gene SNPs (rs11671439, rs2304188, rs8100018, and rs3730051) were studied for estimating the association of the polymorphic gene with PCOD. The PCR products are then purified followed by cycle sequencing reaction which was performed in both directions using Big Dye 1.1 reagent by using the forward or the reverse primer separately. The cycle sequencing product (10 µL) was heated at 95°C for 2 min followed by immediate cooling at 4°C for 2 min in presence of 10 µL formamide. This was followed by capillary run using ABI Prism 310 Genetic Analyzer. The electropherograms of the sequenced DNA samples were analyzed using the BioEdit software and compared with reference sequences submitted in the NCBI database (https://blast.ncbi.nlm.nih.gov).

Statistics

All the polymorphic variants of the four intron regions of the *AKT*2 gene were tested for Hardy-Weinberg equilibrium (HWE) among both the controls and cases for each studied SNP. Logistic regression was used for calculating the Odds Ratios (OR) and 95% confidence intervals (CI) for the polymorphic genotype and alleles among the controls and cases. The Fisher's Exact test was used for calculating the p value. Student's t-test was used for calculating the statistical significance of the differences for the descriptive variables. All the statistical analyses were performed using SPSS v.22 (IBM, USA) software. All the statistical analyses were considered to be significant at $p \leq 0.05$.

RESULT

All PCOD clinical characteristics such as level of FSH and TSH hormone were significantly higher among the cases in comparison to controls except for LH level where it was found to be significantly higher among the cases than in

comparison to the controls (**Table 1**).

All the AKT2 genotypes were found to be in accordance with the HWE (p>0.05). The minor allele frequencies (MAF) for the study subjects were also calculated.

Table 1: Table Representing the Demographic and Clinical Characteristics of the Study Subjects

Characteristics	Control	Case	P value[a]
Age			
Mean ± SD	24.67 ± 6.16	27.78 ± 2.43	0.18
Height			
Mean ± SD	161.27 ± 14.76	105.82 ± 73.44	0.08
Weight			
Mean ± SD	55.31 ± 7.70	65.77 ± 11.57	0.21
LH			
Mean ± SD	18.33 ± 4.32	3.41 ± 2.71	<0.01*
FSH (mIU/mL)			
Mean ± SD	5.77 ± 1.62	9.24 ± 3.69	<0.01*
TSH			
Mean ± SD	4.76 ± 2.21	16.11 ± 5.85	<0.01*
a- Two tailed t-test * p<0.05			

Study from the demographic parameters of the study subjects revealed that there was no significant difference in the age, height and weight of the individuals in the control and case group (**Table 1**).

Table 2: rs11671439 C>T, rs8100018 C>G, rs3730051 A>G, rs2304188 C>T Genotype Frequencies along with Odds Ratio (OR) in Control and Cases

Genotype	Control	Case	OR (95% CI)	P value[a]
rs11671439 C>T				
CC	159	128	1 (Ref)	-
CT	34	66	2.41 (1.50-3.87)	<0.01*
TT	7	6	1.06 (0.36-3.11)	1
rs8100018 C>G				
CC	161	142	1 (Ref)	-
CG	32	45	1.59 (0.96-2.64)	0.075
GG	7	13	2.11 (0.84-5.30)	0.165
rs3730051 A>G				
AA	138	128	1 (Ref)	-
AG	51	53	1.12 (0.71-1.76)	0.645
GG	11	19	1.86 (0.86-4.02)	0.127
rs2304188 C>T				
CC	147	110	1 (Ref)	-
CT	45	78	2.32 (1.49-3.60)	<0.01*
TT	8	12	2 (0.81-4.96)	0.163
a- Fisher's two tailed * p<0.05				

The genotype distribution of the polymorphic genes among the controls and the cases under study are shown in **Table 2**. The odds ratio for the increased risk of PCOD for all the genotypes were determined by taking CC, CC, AA, and CC genotypes as reference for, rs8100018 C>G, rs3730051 A>G, and rs2304188 C>T *AKT2* genes respectively. The rs11671439 CT (OR= 2.41, 95% CI= 1.50-3.87, p<0.01) and rs2304188 CT (OR= 2.32, 95% CI= 1.49-3.60, p<0.01) genotypes were observed to elevate the risk significantly for the disease by 2.41 fold and 2.32 fold respectively.

Genotype combination analysis among rs11671439 C>T, rs8100018 C>G, rs3730051 A>G, rs2304188 C>T polymorphisms on increased risk for disease

Table 3: Distribution and Odds Ratio (OR) of AKT2 double-Combined Genotypes rs11671439 C>T, rs8100018 C>G, rs3730051 A>G, rs2304188 C>T Polymorphisms in Cases and Controls

Genotype	Control	Case	OR (95% CI)	P value[a]
rs11671439 C>T - rs8100018 C>G				
CC-CC	132	99	1 (Ref)	-
CC-CG	23	24	1.39 (0.75-2.59)	0.33
CC-GG	4	5	1.67 (0.47-5.91)	0.507
CT-CC	25	40	2.13 (1.22-3.73)	**0.011***
CT-CG	7	19	3.62 (1.49-8.80)	**<0.01***
CT-GG	2	7	4.67 (1.04-21.00)	**0.046***
TT-CC	4	3	1 (0.24-4.10)	**1**
TT-CG	2	2	1.33 (0.24-7.43)	1
TT-GG	1	1	-	-
rs11671439 C>T - rs3730051 A>G				
CC-AA	111	89	1 (Ref)	-
CC-AG	39	29	0.93 (0.53-1.61)	0.888
CC-GG	9	10	1.39 (0.55-3.48)	0.631
CT-AA	21	34	2.02 (1.10-3.70)	**0.03***
CT-AG	11	24	2.72 (1.28-5.80)	**0.010***
CT-GG	2	8	4.99 (1.12-22.27)	**0.047***
TT-AA	6	5	1.04 (0.32-3.33)	1
TT-AG	1	0	-	-
TT-GG	0	1	-	-
rs11671439 C>T - rs2304188 C>T				
CC-CC	117	65	1 (Ref)	-
CC-CT	36	58	2.90 (1.74-4.84)	**<0.01***
CC-TT	6	5	1.50 (0.46-4.84)	0.532
CT-CC	24	41	3.08 (1.71-5.52)	**<0.01***
CT-CT	9	18	3.60 (1.55-8.35)	**<0.01***
CT-TT	1	7	12.60 (1.73-91.55)	**<0.01***
TT-CC	6	4	1.20 (0.35-4.14)	0.748
TT-CT	0	2	-	-
TT-TT	1	0	-	-
rs8100018 C>G - rs3730051 A>G				
CC-AA	113	94	1 (Ref)	-
CC-AG	40	37	1.11 (0.66-1.87)	0.789
CC-GG	8	11	1.65 (0.65-4.18)	0.342
CG-AA	20	25	1.50 (0.79-2.86)	0.250
CG-AG	9	13	1.74 (0.72-4.16)	0.264
CG-GG	3	7	2.80 (0.76-10.41)	0.194
GG-AA	5	9	2.16 (0.73-6.42)	0.268
GG-AG	2	3	1.80 (0.36-9.14)	0.662
GG-GG	0	1	-	-
rs8100018 C>G - rs2304188 C>T				
CC-CC	120	81	1 (Ref)	-
CC-CT	36	56	2.30 (1.39-3.81)	**<0.01***
CC-TT	5	5	1.48 (0.44-4.96)	0.743
CG-CC	23	23	1.48 (0.78-2.80)	0.249
CG-CT	7	16	3.39 (1.36-8.44)	**0.013***
CG-TT	2	6	4.44 (0.97-20.38)	0.069
GG-CC	4	6	2.22 (0.65-7.62)	0.324
GG-CT	2	6	4.44 (0.97-20.38)	0.069
GG-TT	1	1	-	-

rs3730051 A>G- rs2304188 C>T				
AA-CC	103	74	1 (Ref)	-
AA-CT	29	50	2.40 (1.39-4.13)	**<0.01***
AA-TT	6	4	0.93 (0.27-3.20)	1
AG-CC	37	4	0.15 (0.05-0.44)	**<0.01***
AG-CT	12	21	2.44 (1.14-5.20)	**0.023***
AG-TT	2	24	16.70 (3.94-70.89)	**<0.01***
GG-CC	7	15	2.98 (1.18-7.53)	**0.023***
GG-CT	4	4	1.39 (0.37-5.26)	0.723
GG-TT	0	0	-	-
a- Fisher's two tailed * p<0.05				

Genotype combination analysis of the different *AKT2* polymorphic sites was carried out to determine the role of the polymorphic genes in increasing the risk for the disease (**Table 3**). Cases with rs11671439 CT - rs8100018 CC (OR= 2.13, 95% CI= 1.22-3.73, p =0.011), rs11671439 CT - rs8100018 CG (OR= 3.62, 95% CI= 1.49-8.80, p <0.01), and rs11671439 CT - rs8100018 GG (OR= 4.67, 95% CI= 1.04-21.00, p =0.046) genotype were found to have significant increased risk of 2.13 fold, 3.62 fold and 4.67 fold respectively for the disease. Similarly, cases with rs11671439 CT - rs3730051 AA (OR= 2.02, 95% CI= 1.10-3.70, p =0.03), rs11671439 CT - rs3730051 AG (OR= 2.72, 95% CI= 1.28-5.80, p =0.01), and rs11671439 CT - rs3730051 GG (OR= 4.99, 95% CI= 1.12-22.27, p =0.047), genotype were found to have 2.02 fold, 2.72 fold, and 4.99 fold risk respectively for the disease. Cases with rs11671439 CC - rs2304188 CT (OR= 2.90, 95% CI= 1.74-4.84, p<0.01), rs11671439 CT - rs2304188 CC (OR= 3.08, 95% CI= 1.71-5.52, p<0.01), rs11671439 CT - rs2304188 CT (OR= 3.60, 95% CI= 1.55-8.35, p<0.01) and rs11671439 CT - rs2304188 TT (OR= 12.60, 95% CI= 1.73-91.55, p<0.01) genotype were found to have 2.9 fold, 3.08 fold, 3.6 fold and 12.6 fold increased risk respectively for the disease. The genotype combination of rs8100018 CC - rs2304188 CT and rs8100018 CG - rs2304188 CT was found to have increased risk of 2.2 fold and 3.39 fold for the disease respectively. Similarly individuals with genotypic combination of rs3730051 AA- rs2304188 CT, rs3730051 AG- rs2304188 CC, rs3730051 AG- rs2304188 TT and rs3730051 GG- rs2304188 CC were found to have increased risk of 2.4 fold, 2.44 fold, 16.7 fold and 2.98 fold respectively for the disease.

Table 4: rs11671439 T>C, rs8100018 G>C, rs3730051 A>G, rs2304188 T>C Haplotype Minor Allele Frequencies (MAF) and Odds Ratio (OR) in Control and Cases

SNP	Allele	Overall MAF	Control MAF	Case MAF	OR (95% CI)	P value[a]
rs11671439	**C/T**	0.156	0.126	0.194	1.78 (1.20-2.62)	<0.01*
rs8100018	**C/G**	0.145	0.115	0.174	1.66 (1.11-2.47)	0.016*
rs3730051	**A/G**	0.200	0.182	0.227	1.32 (0.94-1.86)	0.136
rs2304188	**C/T**	0.203	0.152	0.255	1.90 (1.34-2.71)	<0.01*
a- Fisher's two tailed * p<0.05						

The haplotype distribution of the polymorphic genes under study for the controls and the cases are presented in **Table 4**. The OR for the increased risk of PCOD for all the haplotypes were determined by taking C, C, A, and C genotypes as reference for, rs8100018 C>G, rs3730051 A>G, and rs2304188 C>T *AKT2* genes respectively. The rs11671439 T (OR= 1.78, 95% CI= 1.20-2.62, p<0.01), rs8100018 G (OR= 1.66, 95% CI= 1.11-2.47, p=0.016), and rs2304188 T (OR= 1.90, 95% CI= 1.34-2.71, p<0.01) minor alleles were observed to elevate the risk significantly for the

disease by 1.78 fold, 1.66 fold and 1.90 fold respectively.

Table 5: Statistically significant differences in LH, FSH and TSH hormone values for patients bearing various polymorphism of rs11671439 T>C, rs8100018 G>C, rs3730051 A>G, rs2304188 T>C AKT2 gene

Model	Wild-type	Polymorphism	P value[a]
	Mean (SD)	Mean (SD)	
rs11671439 C>T			
LH	3.45 (3.12)	3.34 (1.76)	0.75
FSH	9.90 (3.57)	8.07 (3.64)	**<0.01***
TSH	15.83 (6.10)	17.04 (4.79)	0.12
rs8100018 C>G			
LH	3.42 (3.03)	3.38 (1.67)	0.83
FSH	9.46 (3.65)	8.71 (3.78)	0.31
TSH	16.59 (5.64)	15.46 (5.77)	0.18
rs3730051 A>G			
LH	3.62 (2.98)	3.04 (2.10)	0.11
FSH	9.62 (3.49)	8.57 (3.98)	0.06
TSH	15.82 (6.17)	17.05 (4.64)	0.11
rs2304188 T>C			
LH	3.05 (1.51)	3.86 (3.63)	0.21
FSH	9.63 (3.54)	8.77 (3.84)	**0.04***
TSH	15.94 (6.25)	16.68 (4.91)	0.32
a- Two tailed t-test * p<0.05			

On stratifying the levels of LH, FSH and TSH hormones based on the polymorphic status if AKT2 genes of the patients it was observed that there was a significant decrease in the mean level of FSH hormone among the patients with heterozygous and homozygous recessive genotype of AKT2 rs11671439 C>T and rs2304188 T>C gene in comparison to those with wild type genotype for the same gene (**Table 5**).

DISCUSSIONS

PCOD has been reported to be one of the most common conditions among women especially during the reproductive years (0.6%-92%) (Naether, Baukloh, Fischer, & Kowalczyk, 1994). Previous studies have revealed the significance of insulin, insulin-like growth factor and the associated proteins, sex hormones, growth hormone, LH secretion and binding globulin for the onset of PCOD, yet the complete prognosis of the disease is not fully understood (Insler & Lunenfeld, 1991).

There was an increased mean weight observed among the study subjects in the case group in comparison to control while the mean height of the study subjects in the case group was observed to be lower in comparison to those in the control group. A similar observation was reported by Urman et al., where study subjects with PCOD was observed to have significantly ($p < .05$) higher body mass index in comparison to those in the control group (Urman, Sarac, Dogan, & Gurgan, 1997).

The level of FSH and TSH hormone were significantly higher among the cases than controls. However, the LH level was found to be significantly lower among the cases than in comparison to the controls. In a study by et al., it was observed that there was an elevated LH/FSH ratio was observed among 70.58% women with PCOS (Nath et al., 2019). Banaszewska et al. also reported abnormal LH/FSH ratio among 45.4% of PCOS women (Banaszewska, Spaczyński, Pelesz, & Pawelczyk, 2003). However, in another study by Cho et al., it was reported that LH/FSH ratio has little or no significance in PCOS diagnosis as the median LH/FSH ratio does not alter significantly among the healthy and the PCOS

individuals (Cho, Jayagopal, Kilpatrick, Holding, & Atkin, 2006).

Genotypic analysis from the present research study revealed that the rs11671439 CT and rs2304188 CT genotypes were observed to significantly elevate the risk for the disease by 2.41 fold and 2.32 fold respectively. Haplotype distribution of the polymorphic genes in the study also revealed that the rs11671439 T, rs8100018 G, and rs2304188 T minor alleles were observed to significantly elevate the risk for the disease by 1.78 fold, 1.66 fold and 1.90 fold respectively. Our findings were found to be at par with that reported by Goodarzi et al., where it was observed that rs8100018 were associated with PCOS, and the corresponding haplotype was also associated with PCOS. However, the findings of the rs3730051 was not found to be consistent with the study where a significant association of elevated PCOS risk was reported for individuals with minor allele of AKT2 rs3730051 (Goodarzi et al., 2008). Li et al., in their study, revealed that polymorphism in rs2304186 significantly elevated the risk for PCOS in a Chinese Zhuang population as well as in the non-obesity group (Li, Mo, Sun, Huang, & Wang, 2021). Genotype combination study in increasing the risk for PCOD revealed that there was a significant increase in the risk for PCOD observed upon interaction of rs11671439 C>T, rs8100018 C>G, rs3730051 A>G, rs2304188 C>T *AKT2* gene polymorphisms. Goodarzi et al., observed the effect of combined genotypic interaction for *AKT2* and *GSK3B* gene using additive, and combined logistic regression model. The study demonstrated that combined genotypic interaction between the two genes elevated the PCOD risk among the patients which was not noticed in the presence of single polymorphism which was found to be consistent with our findings indicating that the presence of multiple polymorphism in the AKT2 genes might increase the risk for PCOD in comparison to the presence of individual polymorphism (Goodarzi et al., 2008).

Lastly, our study demonstrated that there was a significant decrease in the mean level of FSH hormone among the patients with heterozygous and homozygous recessive genotype of AKT2 rs11671439 C>T and rs2304188 T>C gene.

The role of family history on the onset of PCOD has been reported previously; however, the genetic etiology of PCOD is not fully understood. This might lead to the fact that there might be some genetic component involved in the onset and prognosis of PCOD which needs to be elucidated. The advent of new sequencing technologies over the years has paved the way for better understanding the genetic components of PCOD which might lead to the identification of novel biomarkers. This research study has been found to be very important as it the first study in the Indian population to elucidate the effect of genetic polymorphism in AKT2 genes along with the gene-gene interaction among the polymorphic sites which might have an important implication for the early diagnosis of women with PCOD along with the estimation of potential risk for developing PCOD complications.

CONCLUSIONS

The study concludes that rs11671439 CT and rs2304188 CT genotypes elevated the risk significantly for the disease by 2.41 fold and 2.32 fold respectively. The rs11671439 T, rs8100018 G, and rs2304188 T minor alleles are significantly associated with the increased risk for the disease by 1.78 fold, 1.66 fold and 1.90 fold respectively. Genotype combination analysis revealed that the presence of multiple polymorphisms in the AKT2 genes might increase the risk for PCOD further in comparison to presence of individual polymorphism.

Authors' Contributions

APS conducted the research work, analysed the data and wrote the manuscript. VDS and CSC analysed data and wrote the manuscript. SM designed the study and wrote the manuscript. All authors read and approved the final manuscript.

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Supplementary Table 1: Primers Sequences used in AKT2 Genotyping Analysis

SNP	Location	PCR Primers
rs2304188	Intron 10	GTCATTGTCCTCCAGCACCT
		TCCAACAGCTGGAAAACCTC
rs11671439	Intron 1	GCAACACTTTGAGGCAGACA
		GGATGGCTTGTTGTGTTGTG
rs8100018	Intron 4	AGAGCGGTGTTGGCTTCTG
		TCAAGAGATCGAGACCATCCT
rs3730051	Intron 8	CGCTGAAGTATGCCTTCCA
		CGGAGGGCTGCTAGGTTTTA